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Surgery of the Rabbit Embryo "in utero"

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SURGERY OF THE RABBIT EMBRYO "IN UTERO"

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I. INTRODUCTION.

In 1925, three authors—Nicholas, Bors and Swenson—published papers suggesting the feasibility of the direct surgical approach to the late mammalian embryo. Nicholas, working with rats, found that it was possible to excise limbs, eyes or tails from 12-20 day embryos "in utero" and still obtain them viable at normal term. Bors, working with rabbits, found that it was possible to excise limbs from 16-25 day embryos and to obtain them viable at 28 days. However, he did not let his operated animals go to term because of the danger that they would be eaten by the mother. Swenson was not interested in the surgery of the embryo but in foetal movement. Previous work on the foetal movements of mammalian embryos had been done without anæsthesia which Swenson considered unsatisfactory. However, it was undesirable to use an anæsthetic which might affect embryonic movement and he describes a technique of ligating the carotid arteries and jugular veins, thus causing anæsthesia by cerebral anæmia. Using this method, he opened the uterus and withdrew the embryos into warm saline during the last third of pregnancy and studied the foetal movements.

In spite of the interesting field that the direct approach to the embryo "in utero" offered, it was not until 1930 that Hooker and Nicholas made use of the technique to investigate regeneration after spinal cord section in the last third of pregnancy of the rat. They reported negative results. Three years later, Larsell (1933) reported substantial reduction in the size of the scapula after limb excision in the 16-19 day embryo.

Quite recently, Hall and Schneiderhan (1945) have reported spinal ganglia hypoplasia in the rat after limb excision during the last third of pregnancy, and Jost (1946), interested in the effect of the embryonic gonads on secondary sex characteristics, has reported successful embryonic castration of rabbits during the last third of pregnancy.

Finally, Wells (1947), also interested in sex problems, has found that the late rat embryo may be brought into the body cavity and still survive provided that the umbilical cord is left attached to the placenta in the uterus.

In spite of the above, little has been described on the details of the technique of the direct approach to the mammalian embryo "in utero," the stage of pregnancy when interference can be successfully carried out and the reliability of the method.

With these points in mind, it seemed worth while to investigate further the possibilities of this method. The rabbit was chosen as the experimental animal and it was decided to remove a limb or the tail from the foetus at various stages of development and to observe the result.

II. TECHNIQUE.

Strict aseptic precautions have been observed in all operations as it is considered that the freedom from infection achieved fully offsets the extra labour involved.

The following procedure has been adopted in making the operations :—

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* This work was carried out while on the staff of the Zoology Department, The University of Melbourne.

A. Preparation (in general laboratory).

1. Inject 0.04 grains of atropine sulphate intravenously (an ear vein is convenient) to prevent excessive mucous formation in the respiratory tract.

2. Strap down on operating table.

3. Shave abdomen.

B. Operation (in operating laboratory).

1. Scrub up and put on gown, cap, mask and gloves with the aid of an assistant.

2. Lay out instruments on a sterile towel. (While this is being done the assistant starts the open ether anæsthesia in the general laboratory and wheels the animal into the operating laboratory.)

3. Swab skin with antiseptic (*e.g.*, Zepharin).

4. Clip on "fenestrated sheet" with towel clips so that the operative area is exposed.

5. Incise skin (2in. incision) in mid line, 1 in. in front of pubic symphysis.

6. Incise muscle layers.

7. Incise peritoneum.

8. Lift out uteri, count embryos present, and have number recorded by assistant,

9. Pack uteri with warm saline packs.

10. Make $\frac{1}{2}$in. incision in uterus and amnion on the anti-mesometrial side. immediately over limb or tail of the embryo.

11. Excise limb or tail and discard.

12. Suture uterus with two fine interrupted cat gut sutures.

13. Remove packs and place uterus back in body cavity.

14. Suture peritoneum and muscle layers with five fine interrupted cat gut sutures.

15. Dust on sulphadiazine powder.

16. Suture skin with five fine interrupted cotton sutures.

III. RESULTS.

In all, nine operations have been carried out on rabbit embryos "in utero," the data on these operations being set out in Table I. in the order in which they were carried out.

TABLE I.

SURGERY OF RABBIT EMBRYOS "IN UTERO."

Operation number	Type of operation	Embryos present	Age at operation	Embryos delivered	Age at delivery
1	Limb excision	3 + X	19 days	3	27 days. Dead
2	Limb excision	7 + X	18 days	7	31 days. Living
3	Caesarean section	6 + 2X	21 days	0	
4	Limb excision	1 + X	21 days	1 + X	30 days. Dead
5	Tail excision	9 + X	21 days	1	30 days. Dead
6	Limb excision	9 + X	22 days	3 + X	31 days. Living
7	Tail excision	8 + X	23 days	8 + X	31 days. Living
8	Caesarean section	6 + 3 X	14 days	0	
9	Caesarean section	6 + 2 X	14 days	0	

N.B.—X indicates embryo operated on or removed.

IV. DISCUSSION.

In operations 3, 8 and 9 it was intended to carry out either a tail or limb excision, but the embryo protruded through the opening in the uterus and had to be removed. Reynolds (1947) points out that the tension on the wall of the uterus is greatest while the conceptus is spheroidal and reaches a maximum at 22 days, just before the conceptus becomes cylindrical. Thus, it is understandable that the embryos tend to protrude if operated on before 22 days. He also points out that there is a preponderance of foetal death when there is maximum tension on the uterus wall, a fact also militating against a high percentage of successful "in utero" operations before this time. Two operations were attempted at 14 days without success; in the second, an attempt being made to relieve the intra-uterine pressure with a syringe before opening the uterus. However, this was unsuccessful.

It remains to be seen if successful operations can be carried out at this stage with the aid of an assistant operator and whether it is possible to operate between 14 and 18 days. Bors (1925) reports a partially successful operation at 16 days in which a badly injured embryo reached the size of a typical 22 day embryo at 28 days.

In operation 1, although a limb excision was carried out, the mother aborted at 27 days, and the operated embryo was apparently absorbed.

In operation 2, although the unoperated embryos were born alive, the operated embryo was apparently absorbed and similarly in operation 5 all but one unoperated embryo were absorbed.

In operation 4, the mother was found dead in her pen with the gut herniated through the body wall incision. The unoperated embryo had been born and the operated embryos were still in the uterus but was of normal 30 day size, indicating that it had lived up to the time of the death of the mother.

A similar gut herniation occurred during the observed parturition of rabbit 6, but three unoperated and the operated embryo were born alive. The most successful experiment was number 7, where all the unoperated and the operated embryos were obtained alive at parturition. Jost (1946) and his school have used this technique in their studies on embryonic sex characteristics in the last third of pregnancy and he gives a brief note on his method not, however, supported by experimental detail. On the other hand, Bors (1925) reports the results of three successful operations at 21 days, but his work differs from the present investigation in that he terminated his experiments at 28 days without letting them run to term.

V. CONCLUSIONS.

It is concluded that it is possible to operate on the rabbit embryo "in utero" after the critical twenty-second day with a reasonable expectation of success, but that before this time the operations become progressively more difficult and less reliable with the technique at present used.

VI. SUMMARY.

The technique of "in utero" operations on the rabbit embryo is described with a discussion of the results obtained at various stages of gestation.

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VII. REFERENCES.

BORS, E. (1925) : Die Methodik der intrauterine Operation am uberlebenden Saugetierfoetus. Arch. Entw. Mech. 115 : 655.

HALL, E. K. & SCHNEIDERHAN, M. A. (1945) : Spinal ganglia hypoplasia after limb amputation in the foetal rat. J. Comp. Neurol. 82 : 9.

HOOKER, D. & NICHOLAS, J. S. (1930) : Spinal cord section in rat foetuses. J. Comp. Neurol. 50 : 413.

JOST, A. (1946) : Sur la différenciation sexuelle de l'embryon de lapin. Remarques au sujet de certaines operations chirurgicals sur l'embryon. Comp. Rend. Soc. Biol. 140 : 461.

LARSELL, O. (1933) : The development of the scapula following intrauterine fore limb amputation in the rat. Anat. Rec. 56 : 57.

NICHOLAS, I. S. (1925) : Notes on the application of experimental method upon mammalian embryos. Anat. Rec. 31 : 355.

REYNOLDS, S. R. M. (1947) : The relation of hydrostatic conditions in the uterus to the size and shape of the conceptus during pregnancy. A concept of uterine accommodation. Anat. Rec. 95 : 283.

SWENSON, E. A. (1925) : The use of cerebral anaemia in experimental embryological studies upon mammals. Anat. Rec. 30 : 147.

WELLS, L. J. (1946) : A method for subjecting foetal rats to laparatomy and repeated subcutaneous injection. Anat. Rec. 94 : 530.